

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-78 (Canceled).

Claim 79 (Currently amended). A tandem fluorescent protein construct comprising:

i) a donor fluorescent protein moiety comprising a contiguous amino acid sequence of 150 amino acids having at least 85% sequence identity with a sequence of SEQ ID NO:2, and which differs from SEQ ID NO:2 by at least a set of amino acid substitutions selected from the group of sets consisting of:

- a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys;
- b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr;
- c) Tyr66His and Tyr145Phe;
- d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys;
- e) Ser72Ala, Tyr145Phe and Thr203Ile; and
- f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr;

characterized in that amino acid residues in said donor fluorescent protein moiety corresponding to positions 65 to 67 of SEQ ID NO:2 are oxidized and cyclized to form a fluorophore; and

ii) an acceptor fluorescent protein moiety comprising a contiguous amino acid sequence of 150 amino acids having at least 85% sequence identity with a sequence of SEQ ID NO:2, and which differs from SEQ ID NO:2 by at least a set of amino acid substitutions selected from the group of sets consisting of:

- a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr; and
- b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr;

characterized in that amino acid residues in said acceptor fluorescent protein moiety

corresponding to positions 65 to 67 of SEQ ID NO:2 are oxidized and cyclized to form a fluorophore, and

iii) a linker polypeptide that couples the donor moiety of i) and the acceptor moiety of ii), wherein the linker polypeptide comprises a protease recognition site and is from 5 to 50 amino acids in length;

wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited[.]; and

wherein the donor moiety, the linker polypeptide, and the acceptor moiety are fused in a single amino acid sequence.

Claim 80 (previously presented). A tandem fluorescent protein construct comprising:

i) a donor fluorescent protein moiety comprising a contiguous amino acid sequence of 150 amino acids having at least 85% sequence identity with a sequence of SEQ ID NO:2, and which differs from SEQ ID NO:2 by at least a set of amino acid substitutions selected from the group of sets consisting of:

a) Tyr66His and Tyr145Phe; and

b) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys;

characterized in that amino acid residues in said donor fluorescent protein moiety corresponding to positions 65 to 67 of SEQ ID NO:2 are oxidized and cyclized to form a fluorophore,

ii) an acceptor fluorescent protein moiety comprising a contiguous amino acid sequence of 150 amino acids having at least 85% sequence identity with a sequence of SEQ ID NO:2, and which differs from SEQ ID NO:2 by at least an amino acid substitution selected from the group consisting of:

a) Ser65Cys; and

b) Ser65Thr,

characterized in that amino acid residues in said acceptor fluorescent protein moiety corresponding to positions 65 to 67 of SEQ ID NO:2 are oxidized and cyclized to form a fluorophore, and

iii) a linker polypeptide that couples the donor moiety of i) and the acceptor moiety of ii), wherein the linker polypeptide comprises a protease recognition site and is from 5 to 50 amino acids in length;

wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited; and

wherein the donor moiety, the linker polypeptide, and the acceptor moiety are fused in a single amino acid sequence.

Claim 81 (Previously presented). A tandem fluorescent protein construct comprising:

A) a donor fluorescent protein moiety comprising:

i) a contiguous amino acid sequence of 150 amino acids having at least 85% sequence identity with a sequence of SEQ ID NO:2, and which differs from SEQ ID NO:2 by at least a set of amino acid substitutions selected from the group of sets consisting of:

a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys;

b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr;

c) Tyr66His and Tyr145Phe;

d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys;

e) Ser72Ala, Tyr145Phe and Thr203Ile; and

f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr;

characterized in that amino acid residues in said donor fluorescent protein moiety corresponding to positions 65 to 67 of SEQ ID NO:2 are oxidized and cyclized to form a fluorophore, or

ii) a contiguous amino acid sequence of 150 amino acids having at least 85% sequence identity with a sequence of SEQ ID NO:2 and comprising a mutation that reduces the hydrophobicity at positions A206, L221 or F223, wherein the mutation attenuates the intermolecular interactions between the donor and acceptor moieties, characterized in that amino acid residues in said donor fluorescent protein moiety corresponding to positions 65 to 67 of SEQ ID NO:2 are oxidized and cyclized to form a fluorophore;

B) an acceptor fluorescent protein moiety comprising:

i) a contiguous amino acid sequence of 150 amino acids having at least 85% sequence identity with the sequence of SEQ ID NO:2, and which differs from SEQ ID NO:2 by at least a set of amino acid substitutions selected from the group of sets consisting of:

a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr; and

b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr; or

ii) a contiguous amino acid sequence of 200 amino acids having at least 85% sequence identity with a sequence of SEQ ID NO:2 and comprising a mutation that reduces the hydrophobicity at positions A206, L221 or F223, wherein the mutation attenuates the intermolecular interactions between the donor and acceptor moieties, characterized in that amino acid residues in said donor fluorescent protein moiety corresponding to positions 65 to 67 of SEQ ID NO:2 are oxidized and cyclized to form a fluorophore, and

C) a linker polypeptide that couples the donor moiety of A) and the acceptor moiety of B), wherein the linker polypeptide comprises a protease recognition site and is from 5 to 50 amino acids in length;

wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited; and

wherein the donor moiety, the linker polypeptide, and the acceptor moiety are fused in a single amino acid sequence.

Claim 82 (Previously presented). The construct of claim 79, wherein the donor fluorescent protein moiety comprises a contiguous amino acid sequence of 200 amino acids having at least 95% sequence identity with the sequence of SEQ ID NO:2 and the acceptor fluorescent protein moiety comprises a contiguous amino acid sequence of 200 amino acids having at least 95% sequence identity with a sequence of SEQ ID NO:2.

Claim 83 (Previously presented). The construct of claim 80, wherein the donor fluorescent protein moiety comprises a contiguous amino acid sequence of 200 amino acids having at least 95% sequence identity with SEQ ID NO:2 and the acceptor fluorescent protein moiety comprises a contiguous amino acid sequence having at least 95% sequence identity with a sequence of SEQ ID NO:2.

Claim 84 (Previously presented). The construct of claim 81, wherein the donor fluorescent protein moiety comprises a contiguous amino acid sequence of 200 amino acids having at least 95% sequence identity with the sequence of SEQ ID NO:2 and the acceptor fluorescent protein moiety comprises a contiguous amino acid sequence of 200 amino acids having at least 95% sequence identity with a sequence of SEQ ID NO:2.

Claims 85 to 90 (Canceled).

Claim 91 (Currently amended). The construct of claim 79, wherein the ~~linker polypeptide comprises a cleavage recognition site for~~ protease is trypsin, enterokinase, HIV -1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, β -secretase for APP, thrombin, renin, angiotensin-converting enzyme, cathepsin D or a kininogenase.

Claim 92 (Currently amended). The construct of claim 80, wherein the protease is ~~linker polypeptide comprises a cleavage recognition site for~~ trypsin, enterokinase,

HIV -1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, β -secretase for APP, thrombin, renin, angiotensin-converting enzyme, cathepsin D or a kininogenase.

Claim 93 (Currently amended). The construct of claim 81, wherein the ~~linker polypeptide comprises a cleavage recognition site for~~ protease is trypsin, enterokinase, HIV -1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, β -secretase for APP, thrombin, renin, angiotensin-converting enzyme, cathepsin D or a kininogenase.

Claim 94 (Currently amended). The construct of claim 82, wherein the ~~linker polypeptide comprises a cleavage recognition site for~~ protease is trypsin, enterokinase, HIV -1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, β -secretase for APP, thrombin, renin, angiotensin-converting enzyme, cathepsin D or a kininogenase.

Claim 95 (Currently amended). The construct of claim 83, wherein the ~~protease is linker polypeptide comprises a cleavage recognition site for~~ trypsin, enterokinase, HIV -1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, β -secretase for APP, thrombin, renin, angiotensin-converting enzyme, cathepsin D or a kininogenase.

Claim 96 (Currently amended). The construct of claim 84, wherein the ~~protease is linker polypeptide comprises a cleavage recognition site for~~ trypsin, enterokinase, HIV -1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, β -secretase for APP, thrombin, renin, angiotensin-converting enzyme, cathepsin D or a kininogenase.

Claim 97 (Previously presented). The construct of claim 79, wherein the donor fluorescent protein fluorescent protein moiety has at least 85% sequence identity with the sequence of SEQ ID NO:2 and the acceptor fluorescent protein fluorescent protein moiety has at least 85% sequence identity with the sequence of SEQ ID NO:2.

Claim 98 (Previously presented). The construct of claim 82, wherein the donor fluorescent protein fluorescent protein moiety has at least 95% sequence identity with the sequence of SEQ ID NO:2 and the acceptor fluorescent protein fluorescent protein moiety has at least 95% sequence identity with the sequence of SEQ ID NO:2.

Claim 99 (Previously presented). The construct of claim 98, wherein the linker polypeptide is from 12 to 40 amino acids in length.